```
CLN00108891_5pv1.a  MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQM----------
NP_003801_NM_003810 MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQMQDKYSKSGIACFLKE
                    ************************************************

CLN00108891_5pv1.a  ----------------------------ILRTSEETISTVQEKQQNISPLVRERGPQ
NP_003801_NM_003810 DDSYWDPNDEESMNSPCWQVKWQLRQLVRKMILRTSEETISTVQEKQQNISPLVRERGPQ
                                                 ********************************

CLN00108891_5pv1.a  RVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGELVIHEKG
NP_003801_NM_003810 RVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGELVIHEKG
                    ************************************************************

CLN00108891_5pv1.a  FYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDAEYGLY
NP_003801_NM_003810 FYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDAEYGLY
                    ************************************************************

CLN00108891_5pv1.a  SIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG
NP_003801_NM_003810 SIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG
                    ****************************************
```

Fig. 1

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```
CLN00493987_5pv1.a          ---------------------------MQMVVLPCLGFTLLLWSQVSGAQGQEFHFGPCQ 33
NP_006841_NM_006850_exon4   ------------------------------------------------------------ 0
NP_006841_NM_006850         MNFQQRLQSLWTLARPFCPPLLATASQMQMVVLPCLGFTLLLWSQVSGAQGQEFHFGPCQ 60
CNL00453866_5pv1.a          ---------------------------MQMVVLPCLGFTLLLWSQVSGAQGQEFHFGPCQ 33
NP_006841_NM_006850_exon1   MNFQQRLQSLWTLA------------------------------------------- 14

CLN00493987_5pv1.a          VKGVVPQKLWEAFWAVKDTMQAQDNITSARLLQQEVLQNVSDAESCYLVHTLLEFYLKTV 93
NP_006841_NM_006850_exon4   ----------------------------------------DAESCYLVHTLLEFYLKTV 19
NP_006841_NM_006850         VKGVVPQKLWEAFWAVKDTMQAQDNITSARLLQQEVLQNVSDAESCYLVHTLLEFYLKTV 120
CLN00453866_5pv1.a          VKGVVPQKLWEAFWAVKDTMQAQDNITSARLLQQEVLQNV------------------- 73
NP_006841_NM_006850_exon1   ------------------------------------------------------------ 14

CLN00493987_5pv1.a          FKNYHNRTVEVRTLKSFSTLANNFVLIVSQLQPSQENEMFSIRDSAHRRFLLFRRAFKQL 153
NP_006841_NM_006850_exon4   FKNYHNRTVEVRTLKSFSTLANNFVLIVSQLQPS------------------------- 53
NP_006841_NM_006850         FKNYHNRTVEVRTLKSFSTLANNFVLIVSQLQPSQENEMFSIRDSAHRRFLLFRRAFKQL 180
CLN00453866_5pv1.a          ---------------------------------SQENEMFSIRDSAHRRFLLFRRAFKQL 100
NP_006841_NM_006850_exon1   ------------------------------------------------------------ 14

CLN00493987_5pv1.a          DVEAALTKALGEVDILLTWMQKFYKL 179
NP_006841_NM_006850_exon4   -------------------------- 53
NP_006841_NM_006850         DVEAALTKALGEVDILLTWMQKFYKL 206
CLN00453866_5pv1.a          DVEAALTKALGEVDILLTWMQKFYKL 126
NP_006841_NM_006850_exon1   -------------------------- 14
```

Fig. 2

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```
CLN00108891_5pv1.a          MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQM---------------- 45
CLN00108891_frag1           ------------------------------------------------------------ 0
CLN00108891_frag2           ----------------------------------------NELKQM---------------- 6
NP_003801_NM_003810_frag1   ------------------------------------------------------------ 0
NP_003801_NM_003810         MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQMQDKYSKSGIACFLKE 60

CLN00108891_5pv1.a          -------------------------------ILRTSEETISTVQEKQQNISPLVRERGPQ 74
CLN00108891_frag1           -------------------------------ILRTSEETISTVQEKQQNISPLVRERGPQ 29
CLN00108891_frag2           -------------------------------ILRTSEETISTVQEKQQNISPLVRERGPQ 35
NP_003801_NM_003810_frag1   ------------------------------------------------VRERGPQ 7
NP_003801_NM_003810         DDSYWDPNDEESMNSPCWQVKWQLRQLVRKMILRTSEETISTVQEKQQNISPLVRERGPQ 120
                                                                                ******* *

CLN00108891_5pv1.a          RVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGELVIHEKG 134
CLN00108891_frag1           RVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGELVIHEKG 89
CLN00108891_frag2           RVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGELVIHEKG 95
NP_003801_NM_003810_frag1   RVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGELVIHEKG 67
NP_003801_NM_003810         RVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGELVIHEKG 180
                            ************************************************************

CLN00108891_5pv1.a          FYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDAEYGLY 194
CLN00108891_frag1           FYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDAEYGLY 149
CLN00108891_frag2           FYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDAEYGLY 155
NP_003801_NM_003810_frag1   FYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDAEYGLY 127
NP_003801_NM_003810         FYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDAEYGLY 240
                            ************************************************************

CLN00108891_5pv1.a          SIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG 235
CLN00108891_frag1           SIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG 190
CLN00108891_frag2           SIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG 196
NP_003801_NM_003810_frag1   SIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG 168
NP_003801_NM_003810         SIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG 281
                            ******************************************
```

Fig. 3

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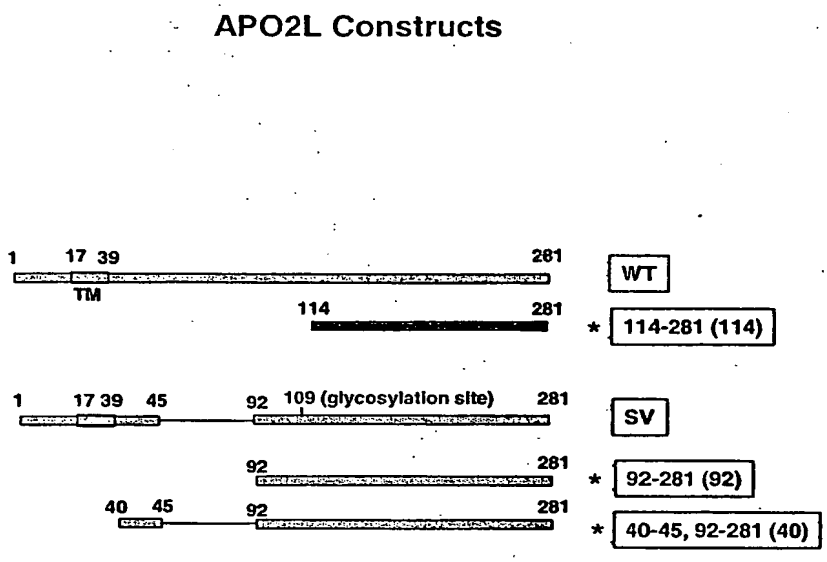
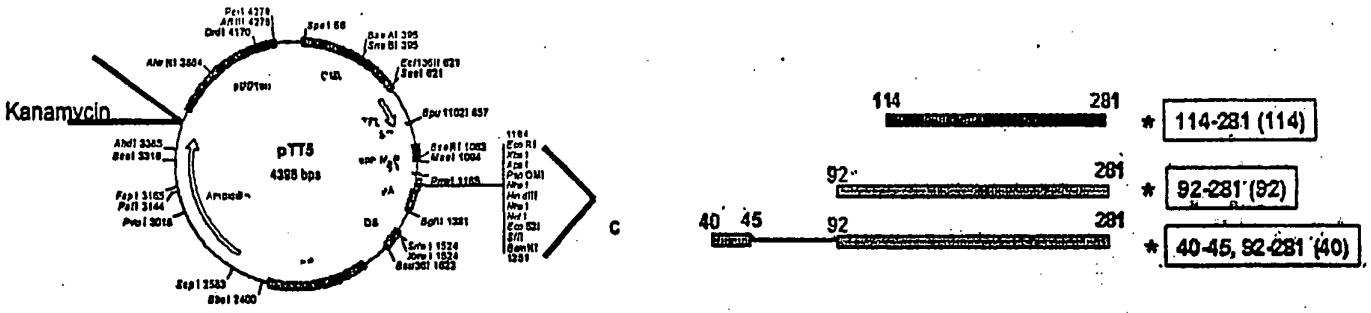

APO2L Constructs
1
17 39
281
TM
WT
114
281
* 114-281 (114)
1
17 39
45
92
109 (glycosylation site)
281
SV
92
281
* 92-281 (92)
40
45
92
281
* 40-45, 92-281 (40)

Fig. 4

Vectors for Producing Secreted Proteins with and w/o a CleavableTag



Vector C:

GCCGCCACCATGAAGACCTGCTGGAAAATTCCAGTTTTCTTCTTTGTGTGCAGTTTCCTGGAACCCTGGGCATCT
Kozak SP
GCAGAATTC——GGATCCCTGGTTCCGCGTGGCTCAGGCTCATTCGAAGGTAAGCCTATCCCTAACCCTCTCCTCGGTCTC
EcoR1 BamH1 Thrombin V5H8
GATTCTACGCGTACCGGTCATCATCACCATCACCATCACCATGGAGGACAGTGA

Our vector in the pipeline:
25 aa for sp and EcoRI---3kD
41aa for BamHI and Thrombin site and V5H8(25aa) —5kD
No-tag: add 3 kD
Tag: add 8 kD

Fig. 5

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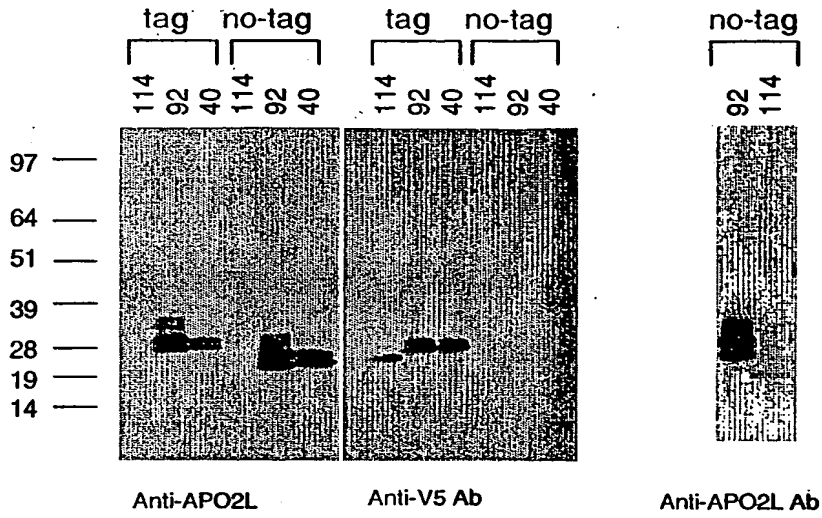
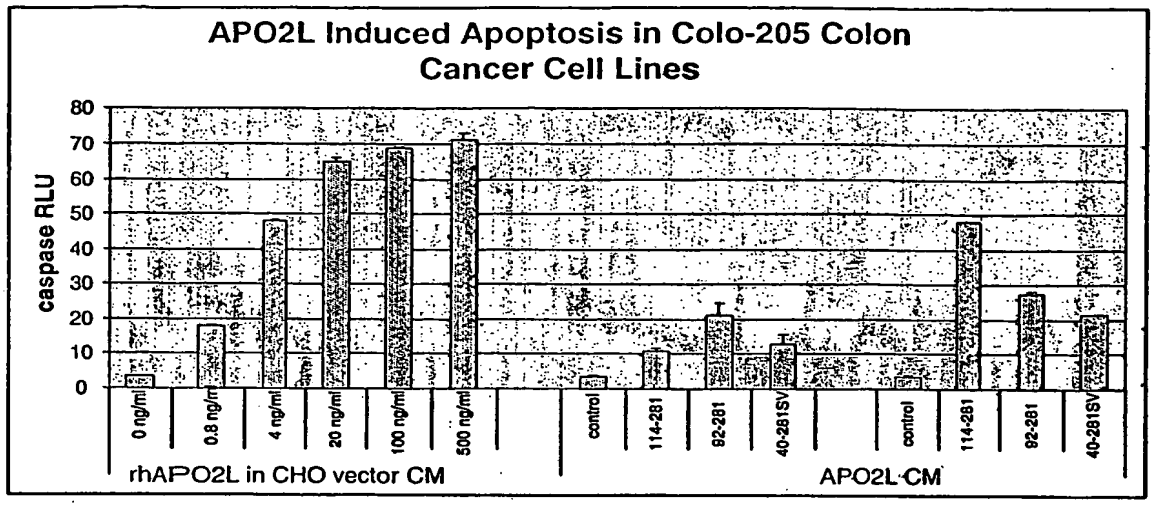


	protein	Molecular Mass	no-tag	with tag
114	168 aa	19.5 kD	23 kD	28 kD
92	190 aa	22 kD	25 kD	30 kD
40	196 aa	22.7 kD	26 kD	31 kD

Fig. 6


APO2L Induced Apoptosis in Colo-205 Colon Cancer Cell Lines
caspase RLU
80
70
60
50
40
30
20
10
0
0 ng/ml
0.8 ng/ml
4 ng/ml
20 ng/ml
100 ng/ml
500 ng/ml
control
114-281
82-281
40-281SV
control
114-281
82-281
40-281SV
rhAPO2L in CHO vector CM
APO2L-CM

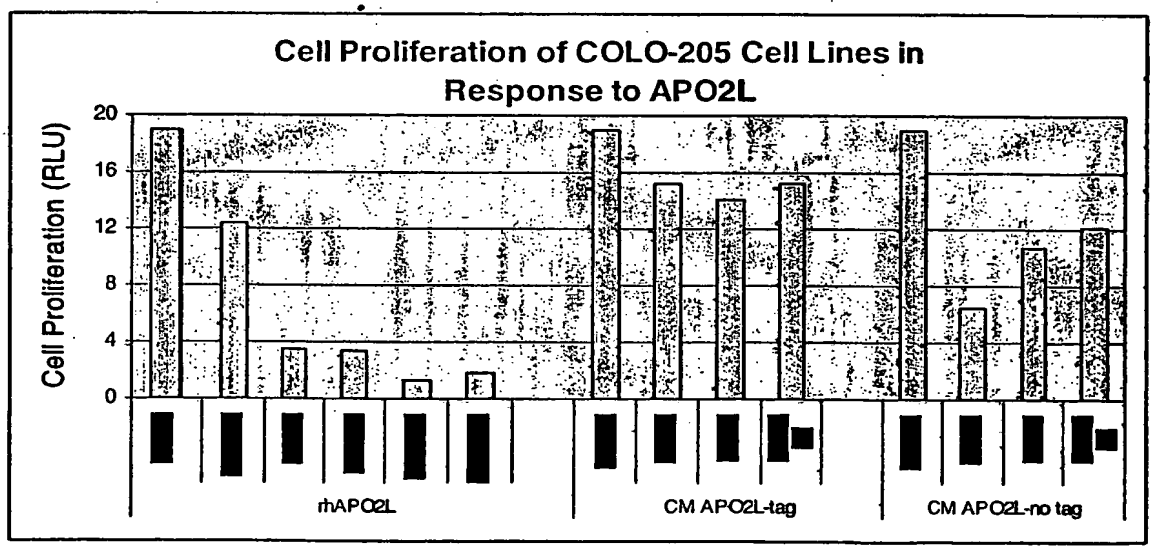
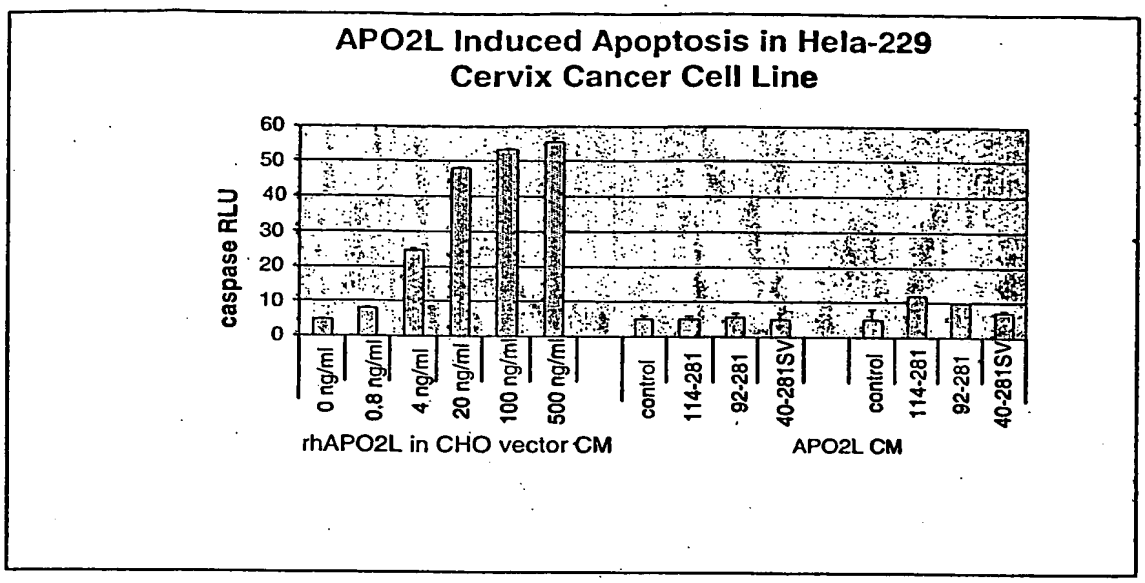

Cell Proliferation of COLO-205 Cell Lines in Response to APO2L
Cell Proliferation (RLU)
20
16
12
8
4
0
rhAPO2L
CM APO2L-tag
CM APO2L-no tag

Fig. 7


APO2L Induced Apoptosis in Hela-229
Cervix Cancer Cell Line
caspase RLU
60
50
40
30
20
10
0
0 ng/ml
0.8 ng/ml
4 ng/ml
20 ng/ml
100 ng/ml
500 ng/ml
control
114-281
92-281
40-281SV
control
114-281
92-281
40-281SV
rhAPO2L in CHO vector CM
APO2L CM

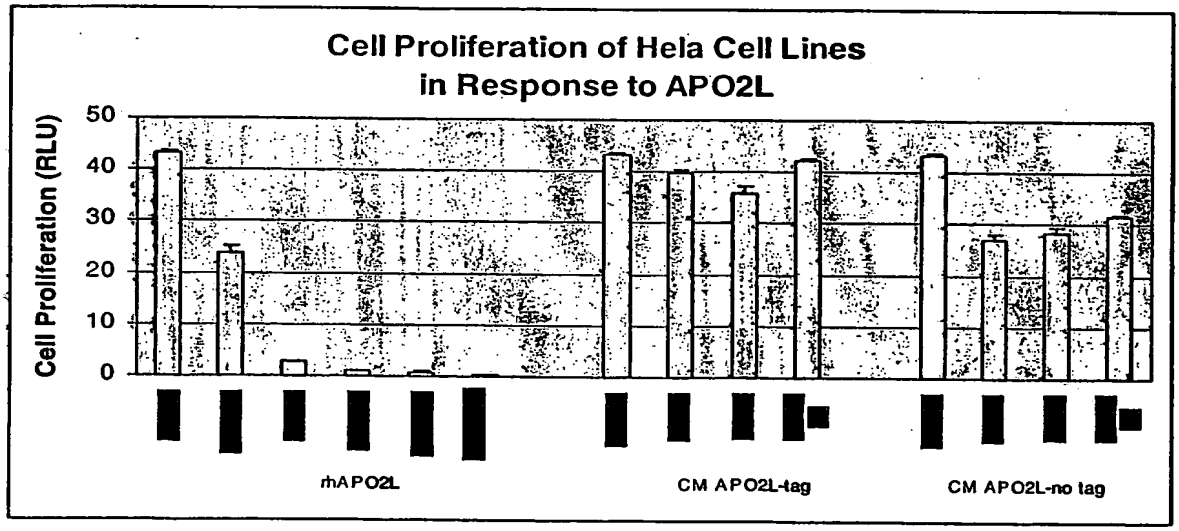

Cell Proliferation of Hela Cell Lines
in Response to APO2L
Cell Proliferation (RLU)
50
40
30
20
10
0
rhAPO2L
CM APO2L-tag
CM APO2L-no tag

Fig. 8

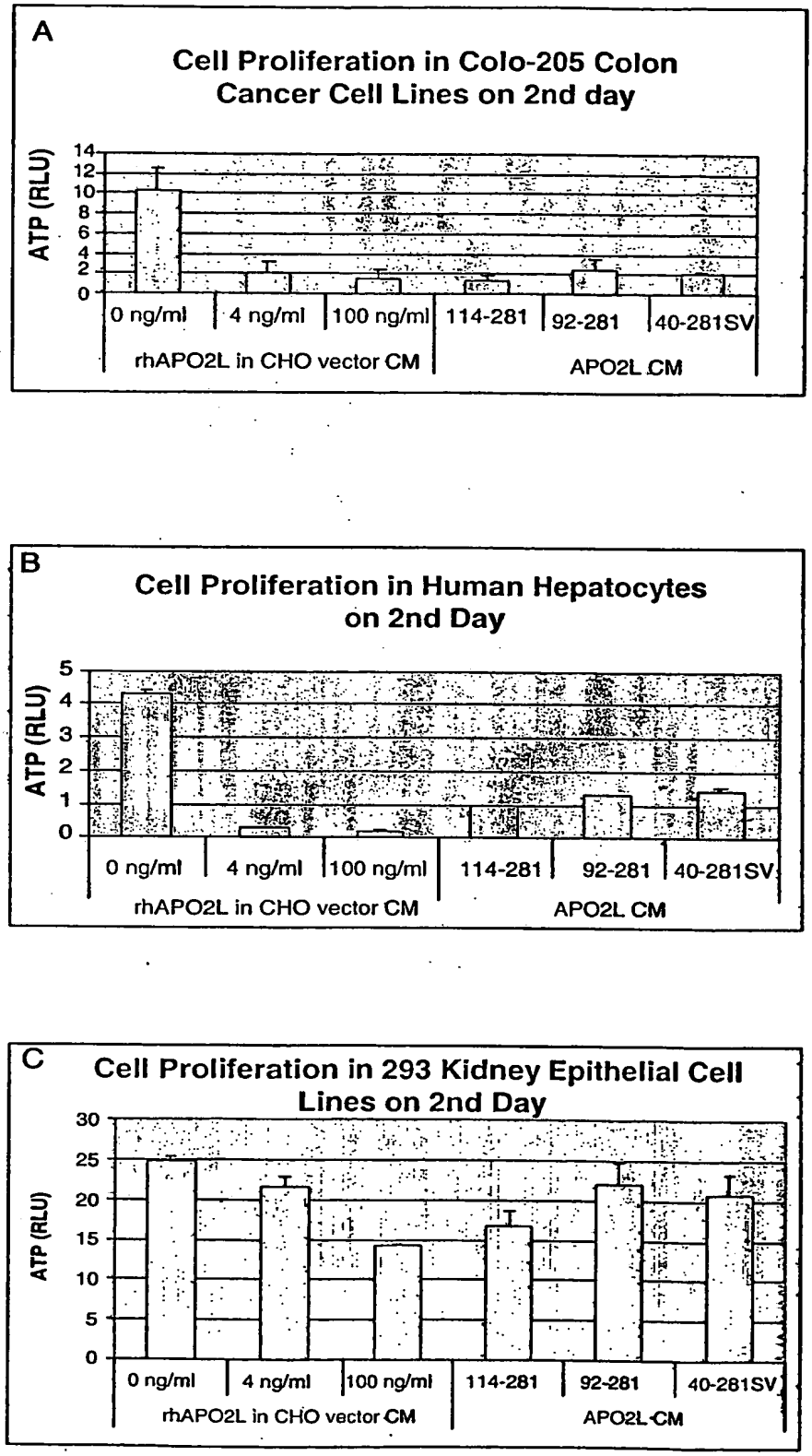
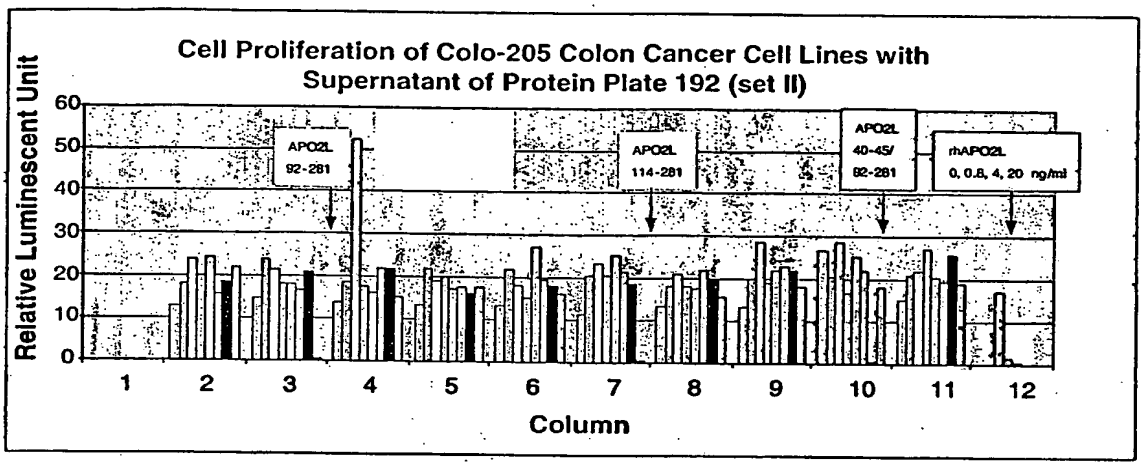

A
Cell Proliferation in Colo-205 Colon Cancer Cell Lines on 2nd day
ATP (RLU)
14
12
10
8
6
4
2
0
0 ng/ml
4 ng/ml
100 ng/ml
114-281
92-281
40-281SV
rhAPO2L in CHO vector CM
APO2L CM
B
Cell Proliferation in Human Hepatocytes on 2nd Day
ATP (RLU)
5
4
3
2
1
0
0 ng/ml
4 ng/ml
100 ng/ml
114-281
92-281
40-281SV
rhAPO2L in CHO vector CM
APO2L CM
C
Cell Proliferation in 293 Kidney Epithelial Cell Lines on 2nd Day
ATP (RLU)
30
25
20
15
10
5
0
0 ng/ml
4 ng/ml
100 ng/ml
114-281
92-281
40-281SV
rhAPO2L in CHO vector CM
APO2L CM

Fig. 9


Cell Proliferation of Colo-205 Colon Cancer Cell Lines with Supernatant of Protein Plate 192 (set II)
Relative Luminescent Unit
60
50
40
30
20
10
0
APO2L 92-281
APO2L 114-281
APO2L 40-45/ 92-281
rhAPO2L 0, 0.8, 4, 20 ng/ml
1 2 3 4 5 6 7 8 9 10 11 12
Column

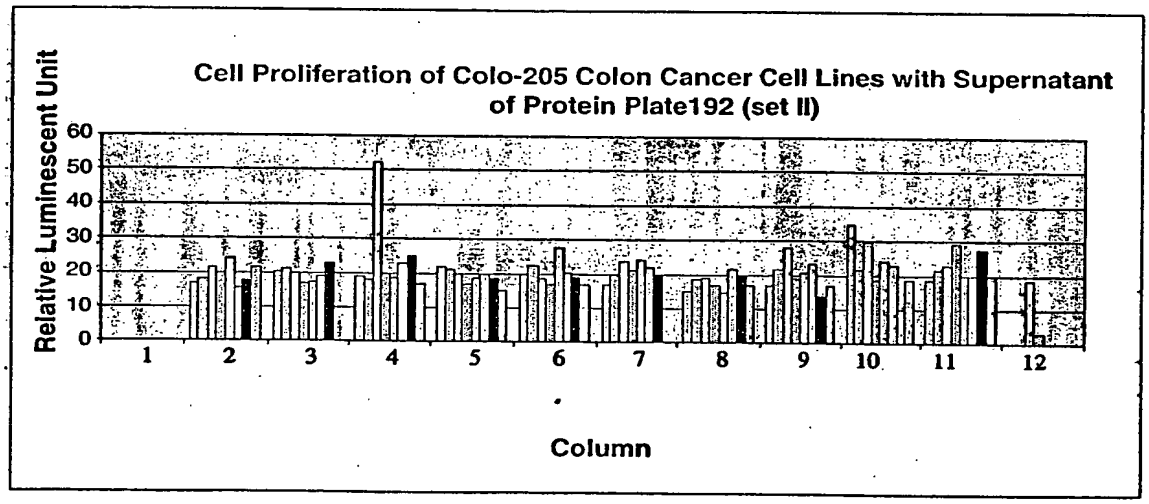

Cell Proliferation of Colo-205 Colon Cancer Cell Lines with Supernatant of Protein Plate192 (set II)
Relative Luminescent Unit
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12
Column

Fig.10

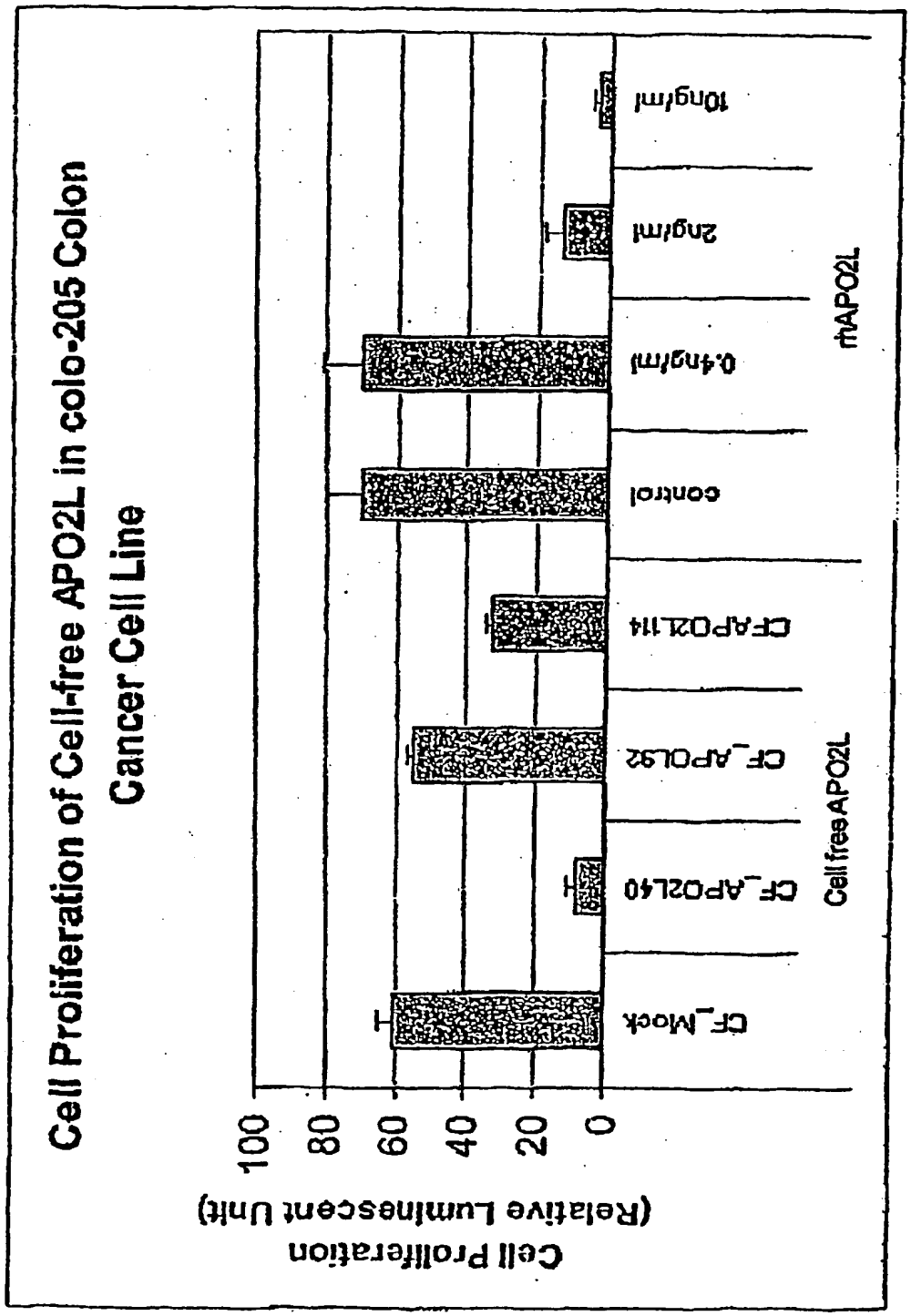

Cell Proliferation of Cell-free APO2L in colo-205 Colon Cancer Cell Line
Cell Proliferation (Relative Luminescent Unit)
100
80
60
40
20
0
CF_Mock
CF_APO2L40
CF_APOL92
CF_APO2L114
control
0.4ng/ml
2ng/ml
10ng/ml
Cell free APO2L
rhAPO2L

Fig. 11

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